UNIVERSITI PUTRA MALAYSIA

CHEMICAL COMPOSITION OF NYPA PALM (Nypa fruticans Wurmb.) VINEGAR AND ITS EFFECT ON ADIPOGENESIS

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FPSK(m) 2019 29

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2018

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chair: Amin bin Ismail, PhD
Faculty: Medicine and Health Sciences

Overweight and obesity are implications of excessive body fat that may give negative effects to health. Nowadays, overweight and obesity are known to be closely related to many types of chronic diseases such as type 2 diabetes, hypertension, coronary heart disease, stroke, and certain cancers. Anti-obesity drug therapy complemented with diet therapy and physical activity has been widely used to treat obesity. However, using the drug as a treatment of obesity could give harmful side effect to the obese patient. Therefore, the objective of this study was to evaluate the chemical composition and anti-obesity effect of nypa palm vinegar (NPV) *in vitro* adipocyte-induced model in comparison to pioglitazone and orlistat as the positive controls. The present study began with the characterization of chemical composition in NPV including organic acids, phenolic compounds, sugar, and alcohol contents. Changes of chemical composition in NPV have been observed base on different fermentation periods and it might be due to naturally presence of microorganisms in *Nypa fruticans* Wurmb. sap. Organic acids and phenolic compounds of fresh nypa sap and its vinegar at different fermentation stages were determined using a cation exchange column of HPLC- DAD. Organic acids detected were acetic acid, lactic acid, succinic acid, tartaric acid, maleic acid, malic acid, quinic acid, oxalic acid, formic acid, and fumaric acid. The prominent organic acid of vinegar was acetic acid in the range of 5-10%. The concentration of acetic acid had significantly increased ($p < 0.05$) from 62.49 ± 0.55 mg/ 100 ml in fresh nypa sap to 2513.80 ± 10.24 mg/ 100 ml, 4510.07 ± 7.03 mg/ 100 ml, and 6036.32 ±5.56 mg/ 100 ml in 4 months, 8 months, and 60 months fermented sap, respectively. Nine phenolic compounds including gallic acid, p-coumaric, o-coumaric, protocatechuic, and chlorogenic acid, catechin, epicatechin, quercetin, and rutin were detected in the samples. The concentration of phenolic compounds significantly diverse ($p < 0.05$) in each sample depend on fermentation periods. Catechin was found the highest concentration in nypa sap (1014.36 ± 21.06 mg/100 ml) and 60 months fermented NPV (3249.40 ± 25.51 mg/ 100 ml) while protocatechuic acid showed the highest concentration in 4 months fermented NPV (1064.50 ± 24.35 mg/ 100 ml) and 8 months fermented NPV (1322.16 ± 7.14 mg/ 100 ml). HPLC-ELSD and GC-FID were employed to quantify sugar and alcohol, respectively. Sugar and alcohol showed significant decrement ($p < 0.05$) in

fermented sap compared to non-fermented sap. DPPH, FRAP and Folin-Ciocalteu assays were used to assess antioxidant capacity in nypa sap and its vinegar. The results indicated that 60 months fermented NPV have the highest antioxidant activity compared to other samples. To assess the NPV effect on OP9 cells viability, MTT assay was carried out and IC_{50}, 2.74% (v/v) of NPV was calculated. IC_{20} value also was determined to represent the non-toxic concentration of NPV for further analysis. Oil Red O (ORO) staining, triglycerides (TG), and glycerol-3-phosphate (G3P) assays were applied to evaluate anti-adipogenesis properties of NPV. After ORO staining, relative lipid accumulation was calculated and NPV-supplementation decreased lipid accumulation in adipocyte up to 57 %. Regardless concentration of treatments, NPV- supplementation showed significant reduction ($p < 0.05$) of TG content compared to non-treated OP9 adipocytes (control). NPV-supplementation caused TG content reduction in the range of 48 - 70 %. Furthermore, 2.0 % (v/v) NPV-supplementation had also suppressed G3P concentration and comparable ($p > 0.05$) to positive control (orlistat), but significantly difference ($p < 0.05$) to pioglitazone. Collectively, the present study has determined bioactive compounds in nypa sap and its vinegar. Potential anti-obesity effects of NPV by *in vitro* study also has been elucidated.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KOMPOSISI KIMIA CUKA NIPAH (*Nypa fruticans* Wurmb.) DAN KESANNYA TERHADAP PEMBENTUKAN SEL LEMAK

Oleh

MOHD ANUAR BIN AB SAMAD

Disember 2018

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Berat badan berlebihan dan obesiti adalah implikasi daripada pengumpulan lemak badan yang berlebihan dan menyebabkan kesan negatif pada kesihatan. Pada masa kini, berat badan berlebihan dan obesiti didapati berkait rapat dengan pelbagai jenis penyakit kronik seperti diabetes jenis 2, tekanan darah tinggi, penyakit jantung koronari, angin ahmar dan kanser. Ubat-ubatan anti-obesiti yang dilengkapi dengan terapi diet dan aktiviti fizikal telah digunakan secara meluas untuk merawat obesiti. Walau bagaimanapun, penggunaan ubat-ubatan sebagai rawatan obesiti boleh memberi kesan sampingan yang berbahaya kepada orang yang obes. Oleh itu, objektif kajian ini adalah untuk menilai komposisi kimia dan kesan anti-obesiti pada cuka nipah dengan menggunakan model sel lemak *in vitro* yang dibandingkan dengan pioglitazone dan orlistat sebagai kawalan positif. Kajian ini bermula dengan pencirian komposisi kimia dalam nira nipah dan cuka nipah termasuk asid organik, sebatian fenolik, gula, dan kandungan alkohol. Perubahan komposisi kimia dalam nira nipah dan cuka nipah telah diperhatikan berdasarkan tempoh penapaian dan perubahan ini mungkin disebabkan oleh kehadiran mikroorganisma semulajadi dalam nira nipah. Asid organik dan sebatian fenolik dalam nira nipah segar dan cuka nipah pada peringkat penapaian yang berbeza telah ditentukan dengan menggunakan kaedah pertukaran kation HPLC- DAD. Asid organik yang dikesan dalam sampel adalah asid asetik, asid laktik, asid sukinik, asid tartarik, asid maleik, asid malik, asid kuinik, asid oksalik, asid formik, asid fumarik. Asid organik yang paling banyak dalam cuka ini adalah asid asetik dalam lingkungan 5-10%. Kepekatan asid asetik telah meningkat dengan signifikan ($p < 0.05$) daripada 62.49 ± 0.55 mg/ 100 ml dalam nira nipah segar kepada 2513.80 ± 10.24 mg/ 100 ml, 4510.07 ± 7.03 mg/ 100 ml, dan 6036.32 ± 5.56 mg/ 100 ml, masing-masing dalam cuka yang diperam selama 4 bulan, 8 bulan dan 60 bulan. Sembilan sebatian fenolik termasuk asid galik, asid p-koumarik, asid o-koumarik, asid protokatekuik, asid klorogenik, katekin, epikatekin, kuersetin dan rutin telah dikesan dalam sampel. Kepekatan sebatian fenolik berbeza dalam setiap sampel bergantung kepada tempoh penapaian. Katekin menunjukkan kepekatan tertinggi dalam nira nipah (1014.36 ± 21.06 mg/100 ml) dan cuka nipah yang diperam selama 60 bulan (3249.40 ± 25.51 mg/100 ml) manakala asid protokatekuic menunjukkan kepekatan tertinggi dalam cuka nipah yang diperam selama 4 bulan (1064.50 ± 24.35 mg/100 ml)

dan cuka nipah yang diperam selama 8 bulan (1322.16 ± 7.14 mg/100 ml). HPLC-ELSD dan GC-FID telah digunakan untuk mengira kadar gula dan alkohol dalam sampel. Gula dan alkohol menunjukkan penurunan yang signifikan ($p < 0.05$) dan ketara dalam cuka nipah berbanding nira nipah segar. Kaedah DPPH, FRAP dan Folin-Ciocalteu telah digunakan untuk mengukur kandungan antioksidan dalam nira dan cuka nipah. Keputusan menunjukkan bahawa cuka nipah yang diperam selama 60 bulan mempunyai aktiviti antioksidan yang paling tinggi berbanding sampel lain ($p < 0.05$). Untuk menilai kesan cuka nipah pada kebolehhidupan sel OP9, ujian MTT telah dilakukan dan nilai IC_{50}, 2.74% (v/v) cuka nipah telah dikira daripada keluk kebolehhidupan sel. Nilai IC_{20} juga telah ditentukan untuk menentukan kepekatan cuka nipah bukan toksik dalam analisis selanjutnya. Pewarnaan Oil Red O (ORO), ujian trigliserida (TG) dan gliserol-3-fosfat (G3P) telah digunakan untuk menilai ciri-ciri anti-adipogenesis cuka nipah. Selepas pewarnaan pada sel lemak, pengumpulan lemak relatif telah dikira dan penambahan cuka nipah dalam sampel telah menurunkan pengumpulan titisan lemak sehingga 57 %. Untuk ujian TG, penambahan cuka nipah sebanyak 2.0 % (v/v) telah menunjukkan pengurangan kandungan TG yang lebih banyak berbanding penambahan cuka nipah yang lebih rendah kepekatannya. Penambahan cuka nipah dalam sampel menyebabkan pengurangan kandungan TG dalam sel lemak dalam lingkungan 48 - 70 %. Tambahan pula, penambahan cuka nipah sebanyak 2.0% (v/v) telah menurunkan kepekatan G3P dalam sel lemak dengan signifikan ($p < 0.05$) dan standing dengan kawalan positif, orlistat. Secara kolektif, penemuan kajian ini telah melaporkan kandungan bioaktif dalam nira nipah dan cuka nipah, malah potensi kesan anti-obesiti cuka nipah dalam kajian *in vitro* telah dijelaskan.

ACKNOWLEDGEMENTS

Apart from my effort, the success of this Master project depends largely from the encouragements and guidelines of many others. First and foremost, I would like to express my gratitude to Allah SWT, The Most Merciful and The Most Gracious, which have given me strength throughout this Master project especially when I was having obstacles and in need of help.

My deepest and sincere gratitude is to my supervisory committee, Professor Dr. Amin Ismail and Associate Professor Dr. Azrina Azlan which guide me without boundary and continuous support during this research study.

I would like to thanks to Mr. Syed Hasbullah Syed Kamaruddin, Mr. Eddy Gadafi, Mr. Simon Md Rawi, and Mrs. Che Maznah Ahmad for the laboratory guidance and technical supervision. Not to forgot, Ministry of Higher Education for MyBrain15 scholarship and Graduate Research Fund, UPM for the financial assistance throughout my research period.

In conjunction, I would also express my deepest gratitude to my beloved parents, family, and friends for their mentally support and financially support and always be with me whenever problems encountered upon completion of this thesis. Last but not least, thousands of thanks to everyone who has directly or indirectly involved in helping me to complete this research study. I really appreciate all help and moral support. Only Allah can repay all the kindness.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as the fulfilment of the requirement for the degree of Master of Science The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

NPV	Nypa Palm Vinegar
HPLC- DAD	High Performance Liquid Chromatography-Diode Array Detector
HPLC-ELSD	High Performance Liquid Chromatography-Evaporative Light Scattering Detector
GC-FID	Gas Chromatography – Flame Ionization Detector
TPC	Total Phenolic Content
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
IC	Inhibition concentration
TG	Triglyceride
G3P	Glycerol-3-Phosphate
WHO	World Health Organization
NHMS	National Health Morbidity Survey
BC	Before Christ
UK	United Kingdom
BMI	Body mass index
RMR	Resting metabolic rate
FAO	Food and Agriculture Organization
NCD	Non-communicable disease
CHD	Coronary heart disease
NHANES	National Health and Nutrition Examination Survey
Hg	Mercury (Hydrargyrum)
SREBP-1	Sterol regulatory element-binding protein 1
mRNA	Messenger Ribonucleic acid
ATP-CL	Adenosine triphosphate citrate lyase
acetyl-CoA	Acetyl coenzyme A
HMG-CoA	5-hydroxy-3-methylglutaryl-coenzyme A
AOX	Alternative oxidase
TVB	Tomato vinegar beverage
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
PV	Pomegranate vinegar
WAT	White adipose tissue
BFR	Body fat ratio
GC	*Garcinia cambogia*
PPARα	Peroxisome proliferator-activated receptor α
PPARγ	Peroxisome proliferator-activated receptor γ
CPT-1a	Carnitine palmitoyltransferase 1A
AMPK	Adenosine monophosphate-activated protein kinase
GR	Gingsam radix
ChREBP	Carbohydrate-responsive element-binding protein
HSL	Hormone- sensitive lipase
PARP	Poly ADP ribose polymerase
AIF	Apoptosis-inducing factor
DNA	Deoxyribonucleic acid

TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
HCl	Hydrochloric Acid
$FeCl_3$	Ferric chloride
$FeSO_4$	Ferrous Sulfate
Na2CO3	Sodium Carbonate
GAE	Gallic acid equivalent
FBS	Fetal bovine serum
MEMα	Minimal essential medium α
CO_2	Carbon dioxide
PBS	Phosphate-buffered saline
DEXA	Dexamethasone
IBMX	3-isobutyl-1-methylxanthine
DM	Differentiation media
OROSM	Oil red o-stained material
SD	Standard deviation
TMS	Trimethyl silyl
RT	Retention time
ANOVA	Analysis of variance
DW	Dry weight
H_2O_2	Hydrogen Peroxide
HClO	hypochlorous acid
CT	Cryptotanshinone
GLUT4	Glucose Transporter 4
GATA2	GATA-binding factor 2 is a transcription factor
TNF-α	Tumor necrosis factor alpha
EGCG	Epigallocatechin Gallate
GPDH	Glycerol-3-phosphate dehydrogenase

CHAPTER 1

INTRODUCTION

1.1 Research background

Nowadays, obesity is a growing health problem and has become a major contributor to mortality and morbidity globally. Indeed, it has become a high-risk factor for many types of chronic diseases such as type 2 diabetes, hypertension, coronary heart disease, stroke, and certain cancers. According to the World Health Organization (WHO, 2016), the worldwide prevalence of overweight and obesity in the adult population aged more than 18 years old are 39% and 13%, respectively. In which, it is over more than 1.9 billion adults were overweight while over 650 million adults were obese. Moreover, WHO has stated that the worldwide prevalence of obesity nearly tripled between 1975 and 2016. In Malaysia, based on previous National Health and Morbidity Surveys (NHMSs) carried out in 2006, 2011 and 2015, an increasing trend of overweight and obesity prevalence was observed among Malaysian adults aged 18 years and older: 29.1% overweight and 14.5% obesity in 2006, 29.4% overweight and 15.1% obesity in 2011, 30.0% overweight and 17.7% obesity in 2015 (Chan et al., 2017).

An obese person may face a psychological and social problem, such as having low self-esteem, difficulty in finding jobs and so on. The risk of life-threatening diseases also increases, particularly cardiovascular disease, type 2 diabetes and certain types of cancer due to obesity. Clinical studies revealed that overweight and obesity are the main causes of high cholesterol level, hypertension, hypertriglyceridemia, glucose, and insulin tolerance.

There have been increasing interest in looking into the effect of natural products and dietary phytochemicals as potential therapeutic agents to treat obesity. Reviews done by (Vermaak et al., 2011; Yun, 2010) have shown varies of natural products contain bioactive compounds such as polyphenol and carotenoid. They have anti-obesity property which can act based on different type of mechanisms. Medicinal plants can reduce weight through five basic mechanisms: controlling appetite, stimulating thermogenesis and lipid metabolism, inhibiting pancreatic lipase activity, preventing adipogenesis, and promoting lipolysis (Kazemipoor et al., 2015). Thus, there have been progress in new dietary supplements, nutraceuticals and functional foods that have anti-obesity effects which are beneficial to health. Vinegar has also been reported its therapeutic properties on cardiovascular risk factors, hyperglycemia, hyperinsulinemia, hyperlipidemia, and obesity (Petsiou et al., 2014).

Production of vinegar involves double fermentation processes which are alcoholic and acetous. Commercial vinegar is produced either by fast or slow fermentation processes. In general, slower methods are used with traditional vinegar, and fermentation proceeds slowly over the course of months or a year. In Malaysia, there is a type of vinegar derived

from *Nypa fruticans* sap. Uniquely, nypa palm vinegar (NPV) is being produced from its sap, not like other vinegar which are derived from the fruits and grains.

Commonly, vinegar consumed by Malaysian community as food condiment is produced artificially. Natural vinegar such as NPV, apple cider vinegar and balsamic vinegar that contain functional bioactive compounds are rarely taken in Malaysian cuisine.

Vinegar is used as both food and medicine in many societies since long time ago (Kadas et al., 2014). During Biblical times, vinegar was used to flavor foods, drunk as an energizing drink, and used as a medicine. In 400 BC Hippocrates indicates vinegar was used medicinally to manage wounds (Conner, 1976).

Vinegar was used as a treatment to attenuate obesity since late 18th century. Post World War 1, antiobesity drugs such as dinitrophenol, amphetamine, and fenfluramine were started to be used and cause many health complications and side effects (Bray, 2014). Vinegar are used as a condiment and traditional medicine worldwide (Bouazza et al., 2016). Thus, scientists have done so many researches investigating the effectiveness of vinegar to be an alternative organic medicine for obesity. It's acidic and phenolic compounds such as chlorogenic acid, gallic acid, and caffeic acid were reported to enhance the lipolysis (Cho et al., 2010).

Triglycerides are water-insoluble lipids consisting of three fatty acids esterified to a glycerol backbone. TG is rapidly hydrolyzed in the capillary beds by lipoprotein lipase, releasing glycerol and free fatty acids, which are absorbed by adipose tissue for storage. The measurement of triglyceride level is useful in the diagnosis of primary and secondary hyperlipoproteinemia, dyslipidemia, and triglyceridemia. TG concentration is also useful in the diagnosis and treatment of diabetes mellitus, nephrosis, liver obstruction, and other diseases involving lipid metabolism or various endocrine disorders (Fredrickson et al., 1967). The most common method to determine triglyceride concentration is by enzymatic hydrolysis of triglycerides to glycerol and free fatty acids followed by either colorimetric or fluorometric measurement of the glycerol released (McGowan et al, 1983; Bucolo & David, 1973).

On the other hand, glycerol-3-phosphate (G3P) is produced either by glycerol via glycerol kinase or by dihydroxyacetone phosphate through glycerol-3-phosphate dehydrogenase (GPDH), and its activity could increase several folds during adipocyte differentiation. In response to cellular signals, G3P can be utilized in multiple pathways: it can be further converted into glyceraldehyde-3-phosphate and enter glycolysis or rapidly generate NAD^+ in brain or muscle tissues through the G3P shuttle or enter the lipid biosynthetic pathway.

1.2 Problem statements

Over a decade ago, world population diets in 1970s started to have dramatic changes toward increasing consumption of processed foods, increases dining out away from home, high consumption of animal foods, edible oils, refined grains, low fiber and added sugar products (Popkin, et al., 2012). Furthermore, due to the modernization of technology, people to have sedentary lifestyles such as television viewing, driving automobiles, reading and many others (Ng & Popkin, 2012). This phenomenon is called a nutrition transition. A study by Zhou et al. (2013) showed increased urbanization has a positive change in nutrition transition which directly gives a positive effect on obesity levels in adults. Popkin (2006) stated changes in nutrition transition have increased energy imbalance and positive shift in body mass index distribution among the adult population.

Moreover, the growing prevalence rates of obesity and overweight in developing countries are advanced higher that developed countries. A systematic review revealed by Khambalia and Seen (2010) showed the trend of overweight and obesity rates in Malaysia has been dramatically increased between the years of 1996 to 2009. This is supported by Baker and Friel (2014) which showed the most population in Malaysia consume high levels of oil and fats, whereas the most population in Thailand and Philippines consume high levels of soft drinks.

Anti-obesity drugs can be classified into two categories which based on different type of action mechanisms. The anti-obesity drugs that are currently used in the market are orlistat and sibutramine. Orlistat is a pancreatic lipase inhibitor which to regulate the gastrointestinal system to reduce fat absorption. Whereas sibutramine is a serotonin and noradrenaline inhibitor, which regulates the central nervous system to suppress appetite. Using these two drugs as a treatment of obesity could give harmful side effects to the obese patient while various natural products could be drug replacer due to their anti-obesity activity (Yun, 2010). A review was done by Rucker et al., (2007) reported that patients who prescribed orlistat treatment have increased rates of gastrointestinal side effect and reduced concentration of high-density lipoprotein while patients who prescribed sibutramine as a treatment of obesity has increased their blood pressure and pulse rate.

Apart from morbidity and mortality burden, obesity also gives a burden in the economic aspects. Wang et al., (2011) reported the projection of obese adult population in the United States (USA) and United Kingdom (UK) by 2030 are more than 65 million and 11 million. The researchers also estimated the combination of the medical cost associated with the treatment of obesity and co-morbidities will increase by $48–66 billion/year in the USA and by £1.9–2 billion/year in the UK by 2030. According to Withrow & Alter (2011), the medical cost of obese individuals is more than 30% greater than normal weight individuals. Thus, alternative actions prior to the treatment should be made as well as effective policies like health promotion and lifestyle intervention can reduce the prevalence of obesity and related diseases which will also give economic benefits to the world.

Due to the undesirable side-effects associated with the currently available anti-obesity medications and limited efficacy, much attention has been focused on developing drugs that directly modulate energy metabolism without affecting the central nervous system. Some natural products such as genistein, epigallocatechin gallate (EGCG), capsaicin, and catechin are known to have anti-obesity effects (Furuyashiki et al., 2014; Hwang et al., 2005). These natural compounds ameliorate obesity either by increasing energy expenditure or by inhibiting adipocyte differentiation.

Vinegar is well known from ancient time and used as a food and medicine product because of its properties (Dogaru et al., 2009; Fushimi & Sato, 2005). All vinegar solutions that primarily contain acetic acid and have been reported to possess physiological effects in human such as blood pressure lowering effects and provide refreshment after exercise (Ou & Chang 2009), antihypertensive properties (Kondo et al., 2001; Nakamura et al., 2010), anticancer effects (Shizuma et al. 2011), improvement of glycogen repletion in liver and muscles (Fushimi & Sato, 2005), reduction of serum cholesterol and triacylglycerols (Fushimi et al., 2006).

Cider vinegar is popular in folk medicine and is suggested as a remedy for various diseases, including obesity and overweight, arthritis, asthma, cough, diarrhea, hair loss, and many other conditions. It can be used as a flavoring agent and a food preservative (Joshi & Sharma, 2009). Mahmoodi et al. (2013) determined the effect of apple vinegar on hematological and blood biochemical factors in type 2 diabetic patients. The results showed that apple vinegar consumption decreased fasting blood sugar glycated hemoglobin, cell volume, and cell hemoglobin, whereas platelets increased. Apple vinegar is reported to have a hypoglycemic effect and may be used in the treatment of type 2 diabetes.

Yamashita et al. (2007) reported that acetic acid is converted to acetate *in vitro* and acetate metabolism by tissues activates adenosine monophosphate-activated protein kinase (AMPK) which play a key role in lipid homeostasis which may explain the lipid-lowering effects of ingested acetic acid in animals. Vinegar consumption also protects from lipid accumulation in liver and skeletal muscle (Yamashita, 2016). Mitrou et al. (2015) reported that vinegar consumption lowers the triglyceride level, whereas no change was observed in non-esterified fatty acid and glycerol in the blood of diabetes mellitus patients.

Worldwide, various kind of natural vinegar such as apple cider vinegar, balsamic vinegar, pomegranate vinegar, and many more have been showed their health-promoting properties. To our concern, there is no study has been reported on the effect of nypa palm vinegar (NPV) in inhibiting adipogenesis and alleviating obesity.

1.3 Significance of the study

Currently, a common anti-obesity drug used is found to have possible side effects to the obese patient (Rucker et al., 2007). It has increased awareness of researchers to seek the safest approach and effective natural treatment, especially from natural sources to treat obesity (Vasudeva et al., 2012). Therefore, this study may provide the evidence base of the uses of nypa palm vinegar (NPV) in treating obesity and limit the usage of the drug as a treatment of obesity.

Vinegar has been found to be effective in weight reduction among the populations (Anastasovska et al., 2009). Acetic acid is the main organic acid present in vinegar and showed numerous health beneficial effects against hyperlipidemia (Beheshti et al. 2012).

Studies have reported that vinegars possess physiological effects in humans such as antihypertensive properties (Nakamura et al., 2010), enhancement of glycogen repletion in liver and muscle (Fushimi & Sato, 2005), anticarcinogenic effects (Baba et al., 2013) and reduction of serum cholesterol and triacylglycerol (Fushimi et al., 2006). Seo et al. (2015) reported that persimmon vinegar reduced body weight and body fat and suppress obesity. Vinegar consumption has been associated with diminished post prandial glucose response following a high glycemic load meal (Johnston & Buller, 2005).

Many underutilized natural products in Malaysia had been reported previously contain high antioxidants with various therapeutic properties. In maximizing utilization of nypa palm, the sap is often used as a beverage, converted to sugar, vinegar, and bioethanol. In fact, consumption of NPV is very minimum, specifically Malaysian even though it has a big potential as an alternative to anti-obesity and other disease medications.

In Malaysia, nypa palm cover almost brackish and swampy area. Nypa sap can be considered very promising for sugar and natural vinegar production. Therefore, the present study was conducted to determine, spread and increase awareness regarding our underutilized natural product and its health benefits.

1.4 Objectives

1.4.1 General objective

To study the chemical compositions of nypa palm vinegar (NPV) and its effect on adipogenesis.

1.4.2 Specific objectives

1. To quantify the organic acid, phenolic compounds, sugar content, alcohol, and antioxidant capacity in NPV.

2. To determine the effect of NPV on OP9 cells viability.

3. To determine the effects of NPV on the lipid accumulation, intracellular triglyceride (TG) content, and glycerol-3-phosphate (G3P) concentration in induced OP9 cells.

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LIST OF PUBLICATIONS

Samad, A., Azlan, A., & Ismail, A. (2016). Therapeutic effects of vinegar: a review. *Current Opinion in Food Science*, *8*, 56-61.

Samad, A., Azlan, A., & Ismail, A. (2016). Health Benefits of *Nypa fruticans* Wurmb. and Its Products, *Functional Foods Wonder Of The World Evidence-Based Functional Foods In Health & Disease,* (pp. 86-102). Serdang, Selangor: UPM Press.

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